Pollen-Derived E_1-Phytoprostanes Signal via PPAR-γ and NF-κB-Dependent Mechanisms[1]

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In a humid milieu such as mucosal surfaces, pollen grains do not only release allergens but also proinflammatory and immunomodulatory lipids, termed pollen-associated lipid mediators. Among these, the E_1-phytoprostanes (PPE_1) were identified to modulate dendritic cell (DC) function: PPE_1 inhibit the DC's capacity to produce IL-12 and enhance DC mediated T_H2 polarization of naive T cells. The mechanism(s) by which PPE_1 act on DC remained elusive. We thus analyzed candidate signaling elements and their role in PPE_1-mediated regulation of DC function. Aqueous birch pollen extracts induced a marked cAMP response in DC that could be blocked partially by EP2 and EP4 antagonists. In contrast, PPE_1 hardly induced cAMP and the inhibitory effect on IL-12 production was mostly independent of EP2 and EP4. Instead, PPE_1 inhibited the LPS-induced production of IL-12 p70 by a mechanism involving the nuclear receptor PPAR-γ. Finally, PPE_1 efficiently blocked NF-κB signaling in DCs by inhibiting IκB-α degradation, translocation of p65 to the nucleus, and binding to its target DNA elements. We conclude that pollen-derived PPE_1 modulate DC function via PPAR-γ dependent pathways that lead to inhibition of NFκB activation and result in reduced DC IL-12 production and consecutive T_H2 polarization.

Pollen allergy is characterized by a predominance of T_H2-biased immune responses to certain proteins, which are released by pollen grains upon contact with the respiratory mucosa. Under physiological exposure conditions, however, allergens are not released alone, but rather in conjunction with pollen granules, starch grains (1), and other, nonprotein substances (2–4). Among these are a number of bioactive lipids (5), the so-called pollen-associated lipid mediators (PALMs).[3] Hitherto existing data point to two main groups of PALMs: First, the immunostimulatory PALMs activating and stimulating cells of the innate immune system such as neutrophils and eosinophils (6, 7) and, second, the immunomodulatory PALMs acting on cytokine and chemokine production of human dendritic cells (DC) skewing the immune systems toward a Type-2 dominated proallergenic immune response (8, 9). The finding that PALMs activate and modulate cells of the immune system added a new, as yet unappreciated aspect to the concept of pollen as mere vehicles of allergens (10).

Besides their well-established role in host defense (11), DCs are involved in hypersensitivity reactions against harmless environmental Ags, the allergens. DCs are not only key players in allergic sensitization (12–14) but even contribute to maintaining and shaping the immune response to allergens in already sensitized individuals (15, 16). Understanding the role of DCs in allergic sensitization has been hampered, however, by the fact that only very few signals have been identified that actively lead to a T_H2-promoting DC phenotype (17–19).

We recently identified pollen-derived phytoprostanes (PPs), the E_1-phyotprostanes (PPE_1), to inhibit DCs' IL12 p70 production and to lead to a T_H2 polarization in naive T cells (8). PPs can be found across a wide variety of plant species and are contained in virtually all organs, including pollen (20). To date, phytoprostanes of the type E_1, B_1, and F_1 were identified in pollen grains (21). Of these, E_1-phytoprostanes compose the most prominent group in aqueous pollen extracts (APE). PPs seem to be involved in plant host defense and are induced by heavy metal exposure and oxidant stress (22, 23). To date, the uptake, receptor employment or signaling pathways of PPE_1 in cells of the human immune system are unknown, which prompted us to analyze the mode of action of E_1-phytoprostanes on human DCs.

PGE_2, a known modulator of DC function, inhibits the secretion of IL-12 p70 via activation of the *E-series* receptors EP2 and EP4 (24, 25). EP2 and EP4 signal via activation of adenylyl cyclase and the induction of cAMP (26). Considering the structural and functional homology of PPE_1 to PGE_2 (21), we hypothesized that PPE_1 may mediate their effect on the IL-12 production of DC via the recognition of EP2 and/or EP4 and the subsequent modulation of the intracellular cAMP response.

Another level at which control of the IL-12 expression might occur is via modulation of the NF-κB pathway regulating the expression of the IL-12 p40 subunit (27). We thus addressed the

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[3] Abbreviations used in this paper: PALM, pollen-associated lipid mediator; DC, dendritic cell; PP, phytoprostane; PPE_1, E_1-phyotprostane; APE, aqueous pollen extract; *Bet.*-APE, aqueous birch pollen extract; moDC, monocyte-derived DC.

question whether the observed inhibition of IL-12 expression by PPE_1 is mediated by interfering with NF-κB signaling.

Herein, we demonstrate that pollen extracts induce a strong cAMP response in DCs that is partially mediated by EP2 and EP4 receptors, suggesting prostaglandin E like ligands in the extract. However, the candidate group of prostaglandin E like substances, the PPE_1, displayed a different profile: it induced a weak cAMP response and the inhibitory effect on IL-12 production was mostly independent of EP2 and EP4. Instead, PPE_1 inhibited the LPS-induced production of IL-12 p70 by a mechanism involving the nuclear receptor PPAR-γ. Further downstream, the signaling seemed to converge again, because both PPE_1 and APE efficiently block the NF-κB signaling cascade by inhibiting IκB-α degradation, nuclear translocation of p65 and binding to its target DNA elements.

Materials and Methods

*Generation of aqueous birch pollen extracts (*Bet.*-APE)*

Pollen grains from white birch (*Betula alba* spec.; Sigma-Aldrich) were incubated at a concentration of 10 mg pollen/ml complete DC medium (RPMI 1640, 10% FCS, 2 mmol/L L-glutamine, 20 μg/ml gentamicin, 500 μmol/L 2-mercaptoehanol) for 30 min at 37°C with vortexing every 10 min. The suspension was centrifuged (10 min, 3000 × *g*) and the supernatant passed through a 0.2-μm pore-size sterile filter (Millipore). For dose response studies, the aqueous extract was diluted in complete DC medium. The concentrations of the *Bet.*-APE given in the text and figures corresponds to the amount of pollen used to generate the extract in a given volume (e.g., 10 mg/ml = extract of 10 mg pollen per ml DC medium).

Phytoprostanes and reagents

PPE_1 and PPF_1 were prepared by autoxidation of α-linolenic acid and purified as described before (21). PGE_2 and 15-deoxy-PGJ_2were purchased at Cayman Chemical. Antagonists of EP2 (AH6809), EP4 (AH23848), and PPAR-γ (GW-9662) were purchased at Sigma-Aldrich. Abs for flow-cytometry were from Becton Dickinson.

Culture of monocyte-derived dendritic cells (moDC)

Healthy, nonatopic volunteers (aged 18–46 years) were screened for total serum IgE levels and for specific IgE against common allergens as described before (8). All blood donors were without medication for at least 15 days before blood sampling. The ethical committee of the Technische Universität Munich approved the study and volunteers were enrolled in the study after written informed consent. PBMC were isolated from peripheral blood by density gradient centrifugation (Lymphoprep, Axis-Shield). $CD14^+$ monocytes were purified by MACS (Miltenyi Biotec) and cultured in complete DC medium at 37°C, 5% CO_2 in the presence of 50 U/ml rhGM-CSF and 50 U/ml rhIL-4 (ImmunoTools). Immature moDC harvested on day 5 were >95% pure as assessed by FACS analysis ($CD14^-$ $CD1a^+$ $HLA\text{-}DR^+$ $CD80^{low}$ $CD83^-$ $CD86^{low}$ $CD40^{low}$) (data not shown).

Stimulation of moDC and measurement of IL-12 p70

For preincubation with EP2-, EP4-, and PPAR-γ antagonists, immature moDC were seeded into six-well plates at a density of 0.7–1 × 10^6 cells/ml complete DC medium plus 50 U/ml rhGM-CSF and 50 U/ml rhIL-4. Cells were treated with the inhibitors AH6809 (30 μmol/L), AH23848 (3 μmol/L), or GW-9662 (0.1 μmol/L). After 1.5 h, the cells were stimulated with 100 ng/ml ultra-pure *Escherichia coli* LPS (Invivogen), either alone or together with different concentrations of PGE_2 (ligand for E-series prostaglandin receptors), 15-d-PGJ_2 (a PPAR-γ ligand), *Bet.*-APE, or PPE_1. Unstimulated moDC served as control. Final concentrations of PGE_2 and PPE_1 were 10^{-8} to 10^{-5} M. Concentrations of *Bet.*-APE are indicated as concentration of pollen used to generate the aqueous extract as described above (range 0.03 to 10 mg/ml). After 24 h, IL-12 p70 and IL-6 were analyzed in cell-culture supernatants by ELISA (human IL-12 p70 and IL-6 matched pair, BD Pharmingen). Viability of the cells after 24 h of culture was determined by propidium iodide staining and was not found to be decreased by any of the stimuli/antagonists at the concentrations used (data not shown).

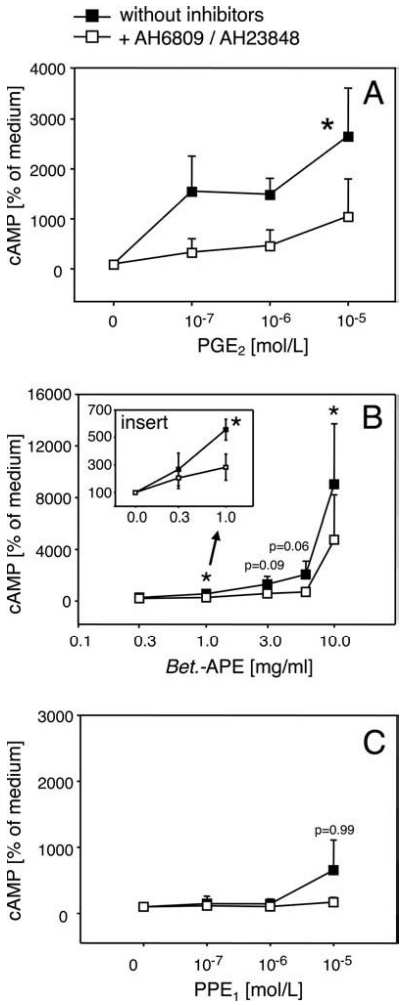

FIGURE 1. Cyclic AMP response in MoDC induced by PGE_2, *Bet.*-APE or PPE_1. MoDC were preincubated for 1.5 h in the absence or presence of EP2- and EP4-antagonists (30 μmol/L AH6809 + 3 μmol/L AH23848) and then stimulated for 40 min with different concentrations of PGE_2 (*A*), *Bet.*-APE (*B*), or PPE_1 (*C*). Cells were lysed and cAMP concentrations were determined in the lysates by ELISA. Results are given as percentage of medium-induced cAMP amounts in lysates (797 ± 272 pmol/L). Results are expressed as mean ± SEM of six independent experiments, each performed in triplicates. *, $p < 0.05$ vs inhibitor-treated sample, Wilcoxon test for paired samples.

cAMP measurements

MoDC were preincubated in the absence or presence AH6809 (30μmol/L) and AH23848 (3 μmol/L) for 1.5 h, then pulsed for 30 min with different concentrations of PGE_2 (10^{-7} to 10^{-5}M), *Bet.*-APE (0.3 to 10 mg/ml), or PPE_1 (10^{-7} to 10^{-5}M). The optimal duration of the pulse had been determined in pre-experiments performing time kinetics. Lysis occurred by addition of an equal volume of lysis buffer supplied within the ELISA kit (cAMP screen, Applied Biosystems). The lysates were directly analyzed for cAMP by ELISA following the manufacturer's instructions.

Preparation of nuclear and cytoplasmic extracts for Western blot analysis and for nonradioactive transcription factor ELISA (Trans-AM)

Cells were rinsed with ice-cold PBS, then resuspended in 200 μl of hypotonic buffer (20 mM HEPES (pH 7.5), 5 mM NaF, 10 μM Na_2MoO_4, 0.1 mM EDTA), incubated for 15 min on ice with vortexing every 2 min, then centrifuged at 340 × *g* for 10 min at 4°C. The resulting supernatants correspond to the cytoplasmic extracts. The obtained pellets were resuspended in 25 μl of hypertonic buffer (20 mM HEPES, 400 mM NaCl, 25% glycerol, 1 mM EDTA, 1 mM EGTA, 0.5 mM Na_3VO_4, and protease

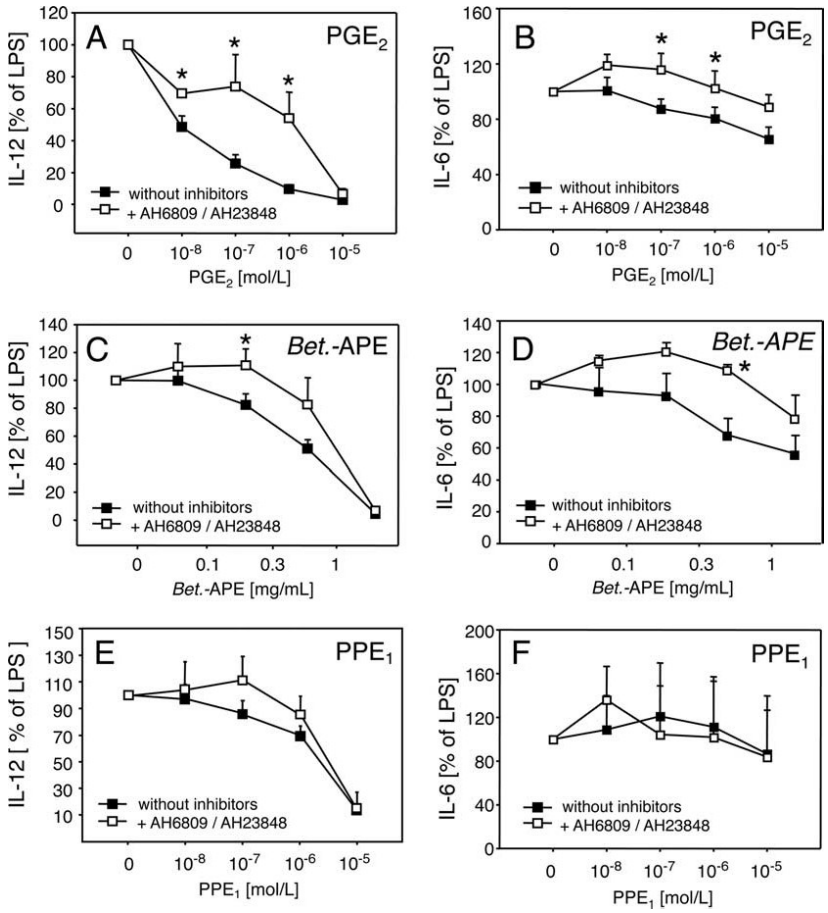

FIGURE 2. PPE_1-induced inhibition of DC IL-12 production is largely independent of EP2 and EP4. MoDC were preincubated for 1.5 h with a combination of two antagonists for the prostaglandin recepotors EP2 (AH6809; 30 μmol/L) and EP4 (AH23848; 3 μmol/L). After the preincubation, *E. coli* LPS (100 ng/ml) and different concentrations of the stimuli PGE_2 (*A* and *B*), *Bet.*-APE (*C* and *D*), or PPE_1 (*E* and *F*) were added to the culture medium. After a further 24 h, supernatants were recovered and amounts of IL-12 p70 and IL-6 were measured by ELISA. Results are given as the percentage (mean ± SEM) of LPS-induced cytokine production (IL-12: 4580 ± 271 pg/ml; IL-6: 71.6 ± 8.8 ng/ml). Depicted are mean values of five (PGE_2), ten (*Bet.*-APE), and five (PPE_1) independent experiments performed with moDC from different donors, each single experiment done in triplicates. *, $p < 0.05$ inhibitor-treated vs noninhibitor-treated sample, Wilcoxon test for paired samples.

inhibitors), incubated on ice for 30 min with vortexing every 5 min, and pelleted by centrifugation at 14000 × *g* for 20 min at 4°C to collect the nuclear extract supernatant. Protein concentrations of both nuclear and cytoplasmic extracts were determined using the Bradford protein assay (Bio-Rad).

Western blot analysis

Samples with equal amounts of protein were loaded on to a 10% acrylamide gel and subjected to SDS-PAGE. The proteins were transferred onto a PVDF membrane and probed with the primary Abs anti-IκBα (sc-371; 1/700) and anti-p65 (sc-372; 1/700), both from Santa Cruz Biotechnology. Immunoreactive bands were revealed with HRP-conjugated secondary Abs using the ECL detection method (Amersham, ECL Western blot analysis system, GE Healthcare).

NFκB p65 transcription factor assay (TransAM method)

The TransAM assay was performed according to the manufacturer's protocol. In brief, TransAM NFκB Kits (Active Motif) contain a 96-well plate on which has been immobilized oligonucleotide comprising the NFκB consensus site (5′-GGGACTTTCC-3′). Nuclear extracts are added to each well and NF-κB binds specifically to the oligonucleotide coated on the plate. Each well is then incubated with primary Ab specific for the active form of bound NF-κB. After incubation with an HRP-conjugated secondary Ab and standard developing solution, the absorbance at 450 nm (reference wavelength 630 nm) was measured in a plate reader (Thermo Scientific) using Revelation software.

Statistical analysis

To reveal statistically significant differences between treatment groups, Wilcoxon test for paired samples was used. *p* values of 0.05 or less were considered to indicate significance.

Results

Bet.-*APE, but not* PPE_1 *induce a strong cAMP response which can be blocked by E-series prostanoid receptor antagonists*

Because PPE_1 bear some structural (21), as well as considerable functional homology to PGE_2, we investigated whether PPE_1 mediate their immunomodulatory effect on moDC via the *E-series* PGE_2 receptors, EP2 and EP4, both of which are known to be functional in DCs and to mediate the PGE_2-induced inhibition of IL-12 production (24). EP2 and EP4 are seven-transmembrane receptors coupled to the activating G-protein subunit Gαs and signal via induction of the second messenger cAMP. Therefore, we first analyzed whether *Bet.*-APE or PPE_1 were able to elicit a cAMP response in DC. The functionality of the assay was confirmed by the observation that PGE_2 induced the formation of cAMP, an effect that was attenuated significantly in the presence of inhibitors for the PGE_2 receptors EP2 (AH6809) and EP4 (AH23848) (Fig. 1*A*). *Bet.*-APE stimulation lead to a strong, dose-dependent cAMP induction, which, as observed before, was 3- to 4-fold higher than that elicited by PGE_2 (9). The cAMP induction elicited by *Bet.*-APE was attenuated in the presence of the EP2 and EP4 antagonists (Fig. 1*B*).

PPE_1 failed to induce a significant cAMP response, although at the concentration of 10^{-5} M there was some formation of cAMP above background level. However, no dose-response relationship could be observed, and the inhibition of cAMP formation in the presence of AH6809 and AH23848 did not reach statistical significance (Fig. 1*C*).

PPE_1 *inhibit the LPS-induced IL-12 production by a mechanism largely independent of EP2 and EP4 receptors*

We next analyzed whether the inhibition of the LPS-induced IL-12 secretion by *Bet.*-APE or PPE_1 was dependent on the activation of PGE_2 receptors EP2 and EP4. To that purpose, DC were stimulated with LPS and different concentrations of PGE_2, *Bet.*-APE, or PPE_1 in the absence or presence of EP2 and EP4 antagonists. The dose-dependent inhibitory effect of PGE_2 on the LPS-induced IL-12 production was reversed significantly in

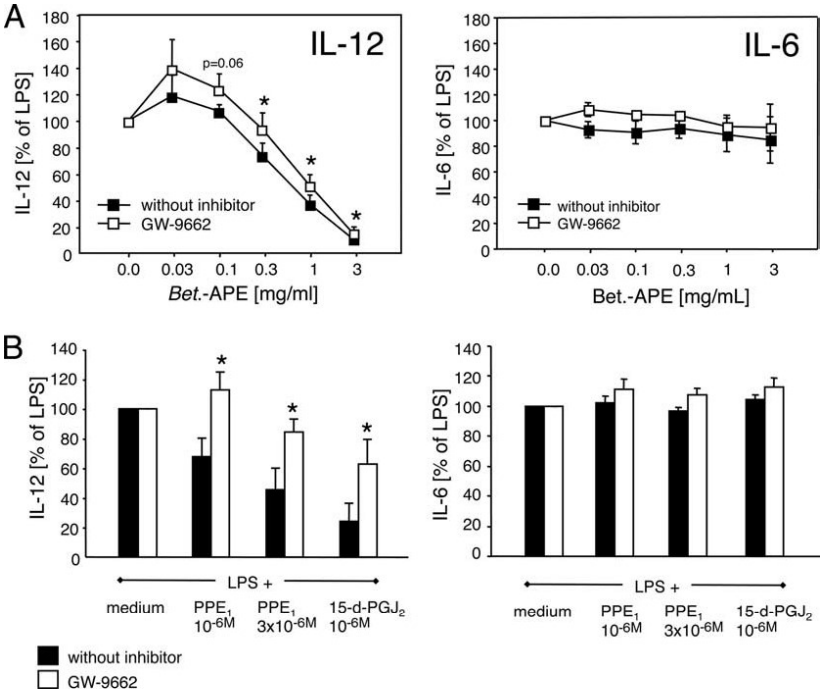

FIGURE 3. Inhibition of IL-12 p70 by PPE_1 depends on PPAR-γ. MoDC were preincubated for 1.5 h with the selective PPAR-γ antagonist GW-9662 (0.1 μmol/L) and then stimulated with *E. coli* LPS (100 ng/ml) plus different concentrations of (*A*) *Bet.*-APE or (*B*) 15-d-PGJ_2, or PPE_1. After a further 24 h, supernatants were analyzed for the presence of IL-12 p70 and IL-6 by ELISA. Results are given as the percentage (mean ± SEM) of LPS-induced cytokine production (4735 ± 492pg/ml; IL-6: 86.83 ± 9.5 ng/ml). Data are mean values (±SEM) of five independent experiments, each performed in triplicates. *, $p < 0.05$ inhibitor-treated vs noninhibitor-treated sample, Wilcoxon test for paired samples.

the presence of the EP2- and EP4-antagonists (Fig. 2*A*). Like PGE_2, *Bet.*-APE and PPE_1 inhibited the LPS-induced IL-12 response (as previously shown in Ref. 8), an effect which was only partially reversed in the presence of the EP2/EP4-antagonists, as illustrated by the slight shift of the dose-response curves to the upper right (Fig. 2, *C* and *E*).

In parallel to IL-12 production, the LPS-induced secretion of the proinflammatory cytokine IL-6 was measured. PGE_2 and *Bet.*-APE both induced a dose-dependent decrease in IL-6 secretion (Fig. 2, *B* and *D*), which was, however, not as pronounced as in the case of IL-12 and not statistically significant in the case of *Bet.*-APE. In the presence of the EP2/EP4-antagonists, the dose-response curves shift significantly rightward (Fig. 2, *B* and *D*). PPE_1 did not inhibit IL-6 secretion, and there was no effect of the EP2/EP4-antagonists (Fig. 2*F*).

PPE_1 inhibit the IL-12 production of DC by a mechanism involving nuclear receptor PPAR-γ

The cyclopentenone arachidonic acid metabolite 15-d-PGJ_2 inhibits DC IL-12 production by activating the intracellular receptor/transcription factor PPAR-γ (28). To address whether similar mechanisms may be effective when DC are exposed to pollen derived lipids we analyzed the effect of PPE_1 or APE on the production of IL-12 and IL-6 in the presence or absence of a selective PPAR-γ antagonist (GW-9662). The known PPAR-γ agonist 15-d-PGJ_2 served as control. In the presence of the PPAR-γ antagonist, the inhibitory effect of *Bet.*-APE on IL-12 production was attenuated significantly albeit only mildly over a wide range of concentrations (Fig. 3*A*). In contrast, the inhibitory effect of 15-d-PGJ_2 and PPE_1 on IL-12 production was reversed almost completely when PPAR-γ activation was blocked by GW-9662 (Fig. 3*B*).

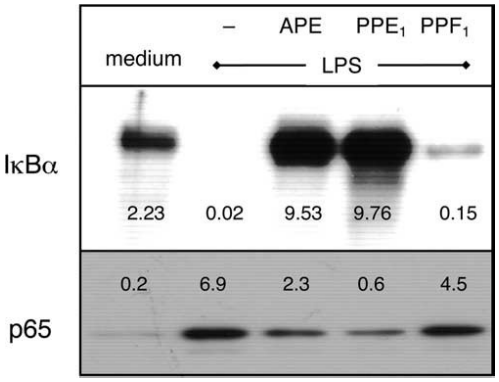

FIGURE 4. PPE_1 affect the NFκB pathway in mature DC, whereas PPF_1 does not. DC were pretreated with PPE_1 (10^{-6} M), PPF_1 (10^{-6} M) or *Bet.*-APE (10 mg/ml) for 30 min at 37°C, and afterward they were cultured with LPS (100 ng/ml) for 20 min. After treatment, cytosolic and nuclear proteins were subjected to SDS-PAGE followed by immunoblotting using respectively anti-IκBα (Santa Cruz Biotechnology, sc-371) and anti-p65 (Santa Cruz Biotechnology, sc-372). Equal loading of protein was controlled by Comassie blue staining of the membranes.

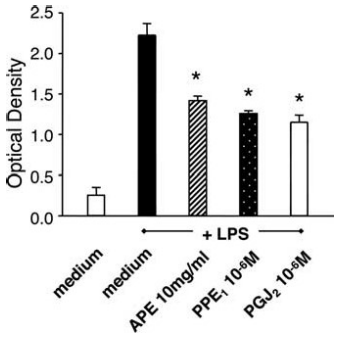

FIGURE 5. PPE_1 inhibit LPS-induced NFκB DNA binding in DC. DC were preincubated with PPE_1 (10^{-6} M), *Bet.*-APE (10 mg/ml) and PGJ_2 (10^{-6} M) for 30 min at 37°C, subsequently stimulated with LPS (100 ng/ml) for 20 min, and lysed. Afterward, 2 μg of nuclear cell lysate were loaded on a 96-well plate precoated with the oligonucleotide containing the NFκB consensus site. Results represent mean ± SD of three independent experiments. *, $p < 0.05$, Wilcoxon test for paired samples.

PPE_1 inhibit NF-κB signaling

Because the nuclear receptor PPAR-γ interferes with the NFκB pathway, we analyzed the ability of phytoprostanes E_1 and $-F_1$ and *Bet.*-APE to modify NFκB activation. PPF_1 neither blocked the LPS-induced IL-12 p70 production nor any other DC effector function investigated (8) and thus served as negative control. PPE_1 but not PPF_1 strongly inhibited the LPS-induced degradation of IκBα in DC and, at the same time, inhibited the translocation of NFκB-p65 subunit to the nucleus (Fig. 4). The same effect was observed for *Bet.*-APE known to contain substantial amounts of PPE_1 (8). We further analyzed the capacity of PPE_1 to interfere with the LPS-induced NFκB DNA binding activity by performing a TransAM assay. In this study, we observed that PPE_1 down-regulated the LPS-induced NFκB DNA binding activity, as did *Bet.*-APE and the positive control, PGJ_2 (Fig. 5).

Discussion

In this study, we provide first details on the signal transduction mechanisms induced by pollen-derived water soluble factors in human DC. First, we demonstrate significant cAMP induction by *Bet.*-APE, an effect that is attenuated in the presence of EP2- and EP4- antagonists. PPE_1 does not show such effect. In concordance with this, *Bet.*-APE, but not PPE_1, inhibit the LPS-induced IL-12 production of DC partly by a mechanism depending on the E-series prostanoid receptors EP2 and EP4. We further show that PPE_1 mediate the IL-12 inhibition via a mechanism involving the nuclear receptor PPAR-γ and by interfering with NF-κB signaling.

As previously shown (9), *Bet.*-APE strongly and dose-dependently induced intracellular formation of cAMP. We now provide pharmacological evidence that this cAMP induction depends partly on *E-series* prostanoid receptors like EP2 and/or EP4. Stimulation of DC with PPE_1 did not yield as clear-cut results. Although the observed strong cAMP inducing activity of *Bet.*-APE likely has to be ascribed to substance(s) different from PPE_1, it remains to be determined whether other PALM(s) or protein(s) mediate this effect. We could recently show that *Bet.*-APE, much like PGE_2, enhance the migration of LPS-stimulated DC toward the chemokines CCL19, CCL21, and CXCL-12, an effect that was abrogated in the presence of an adenylyl cyclase inhibitor (9). Moreover, incubation of DC with LPS plus *Bet.*-APE, but not with PPE_1, lead to enhanced secretion of the T_H2-attracting chemokine CCL17 (9). Last, but not least, we only recently demonstrated that in vivo, intranasal instillation of *Bet.*-APE lead to a T_H2 shift in draining lymph node T cells, while intranasal E_1-phytoprostanes down-regulated both T_H1 and T_H2 cytokine production (29).

Although the PGE_2- and *Bet.*-APE-mediated inhibition of the LPS-induced IL-12 secretion was significantly reversed in the presence of EP2- and EP4-antagonists the PPE1-mediated IL-12 inhibition was just slightly, not significantly, attenuated in the presence of the EP2/EP4-antagonists.

It is well established that PGE_2 mediates its inhibitory effect on the TLR-ligand-induced IL-12 production via the $G_{\alpha s}$-coupled receptors EP2 and EP4 (24), and our present data are in good agreement with these findings, although the pronounced cAMP response to *Bet.*-APE probably cannot be ascribed to an activation of EP2 and EP4 alone.

PPE_1, but not PPF_1 were able to inhibit IκBα degradation in the cytoplasm, p65 translocation to the nucleus and NF-κB binding to κB binding sites on the DNA. This suggests that the PPE_1-mediated inhibition of the LPS-induced IL-12 production is due to a block of NF-κB signaling, resulting in reduced transcription of the IL-12 p40 subunit gene. Because NF-κB signaling and cAMP pathway act independently from another, this observation corresponds well to the aforementioned divergent effects of *Bet.*-APE and PPE_1 on cAMP levels and EP2/EP4 activation. Functionally, NF-κB binding to its target DNA sequences was inhibited in a TransAM assay by PPE_1, *Bet.*-APE, and the positive control PGJ_2. For the cyclopentenone PGJ_2, direct as well as indirect inhibition of NF-κB-dependent gene transcription have been described (summarized in Ref. 30). Recent evidence suggests that cyclopentenone PPs PPA_1 and d-PPJ_1 in dietary oils might exert anti-inflammatory effects by inhibiting NF-κB activity (31). Direct inhibition of NF-κB signaling by cyclopentenones is achieved by an attack mediated by an electrophilic carbon in the cyclopentenone ring system toward sulfhydryl-groups in exposed cysteine-residues of the IκB kinase and the NF-κB DNA-binding domains (32). A different mechanism by which cyclopentenone PGs like the endogenously formed PGD_2 metabolite15-d-PGJ_2 interfere with NF-κB signaling is by binding to the nuclear receptor PPAR-γ. Ligand-induced activation of PPAR-γ leads to the transcriptional activation of PPAR-γ-responsive genes as well as the transrepression of NF-κB-dependent genes (30).

Importantly, the inhibitory effect of PPE_1 on the LPS-induced IL-12 production of DC was reversed almost completely when PPAR-γ activation was inhibited. This supports the idea that the intracellular receptor PPAR-γ is involved in the PPE_1-mediated IL-12 inhibition and is in accordance with the results obtained for NF-κB signaling. Interestingly, activation of PPAR-γ in murine DC has recently been linked with the induction of $CD4^+$ T cell anergy (33). A possible activation of PPAR-γ by PPE_1 might explain the divergent behavior of PPE_1 and *Bet.*-APE in the mouse model (29).

In summary, the results of our study show that pollen-derived PPE_1 interfere with NF-κB signaling and inhibit IL-12 via a PPAR-γ dependent mechanism. Although we were able to dissect one group of lipid mediators and elements of their signaling pathway, it is very likely that additional bioactive substances contribute to the strong T_H2-polarizing effect of *Bet.*-APE both in vitro and in vivo. This assumption is supported by the strong induction of cAMP by the pollen extract (as compared with PPE_1) that can partially be blocked by EP2 and EP4 antagonists. Aqueous birch pollen extracts thus seem to contain as yet unidentified "PGE_2-like" substance(s) which signal via EP2/EP4. Because the EP2-/EP4 antagonists only partially blocked the *Bet.*-APE induced cAMP response, there seem to be additional substances involved that may act via binding to different cAMP dependent receptors. PPE_1, even though structurally homologous to PGE_2, do not seem to act via EP2 and EP4 and fail to elicit a strong cAMP response but instead inhibit IL-12 via a mechanism depending on PPAR-γ. It seems likely that several different pollen-derived factors synergize and account for the pronounced immunomodulatory capacity of *Bet.*-APE. This might explain the diverging effects of PPE_1 and *Bet.*-APE in the animal model (29). It is a worthwhile challenge to identify some of the other bioactive factors released from pollen and to analyze their effects on the outcome of immune responses. These future studies will add to our understanding of allergic sensitization to pollen and ultimately pave the path for new strategies in the therapy of pollen allergy.

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Disclosures

The authors have no financial conflict of interest.

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